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PROSPECTS FOR A GENETICS OF SOMATIC AND TUMOR CELLS*

(Discussion of Doctor G. Klein's Paper)

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Since I have had no experience at all with ascites tumors, Doctor Theodore S. Hauschka's suggestion that I discuss Doctor Klein's paper had to be construed as an invitation to review the potential impact of microbial genetics on tumor studies. In fact, most of the hypothetical notions that will be summarized are the result of informal discussions with Doctor Hauschka, Doctor Klein, and others. The analogy between the ascites tumor cell and an infectious microorganism hardly needs to be stressed, and something may be gained by pursuing the analogy both in method and in concept.

Bacteria have been the subject of investigation of several types of genetic change and exchange:

Mutation and selection. Klein's paper illustrates one of the most disputed problems of bacterial genetics, the origin of adaptive mutations, usually involving resistance to drugs but also including nutritional or other biochemical modifications. By ever more precise methods, it has become possible to prove that such adaptive mutations occur "spontaneously," that is, independently of the environmental stress to which they confer improved fitness.[1, 2] By indirect selection,[3] for example, drug-resistant bacteria can be isolated without exposing them to the drug. It would be possible, if tedious, to extend this technique to tumor cells, but I doubt whether such an extension beyond these already impressive demonstrations[4] would be worth the effort that might be expended on more informative experiments.

Once given reliable procedures for the detection and estimation of mutations, we may then anticipate a more systematic application of mutagenic agents, both radiations and chemicals: at first, to facilitate the isolation of mutants that might be useful as genetic markers and, later, for the elucidation of radiobiological effects on tumor cells—the role of genetic damage in radiation injury[5, 6] and the mechanism of radioresistance, be it polyploidy[6] or an adaptive mutation itself.[7]

The role of selection in the determination of cell populations is obvious in some of Klein's and Hauschka's studies.[8] Bacteriological studies[9] have also drawn attention to the sampling effect of adaptive mutations: their origin, usually from a single cell, means that genotypes in mixed populations can become randomly fixed as effectively as by the purposeful technique of single cell isolation.

The impact of selective forces should not be deduced solely on hypothetical grounds, as many examples of anomalous selection in mixed cultures are not readily predictable from the behavior of the isolated components.[10]

Transduction.[11] The availability of a variety of genetic markers has led to

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a more thorough analysis of the famous "pneumococcus transformation" of Griffith, which has been followed by a series of examples of genetic exchange best understood as genetic transduction, namely, that fragments of chromosomes (or exogenotes) may be transferred from one cell to another. The remarkable feature of transduction is the ability of the fragment not only to survive its transportation but to replace homologous genes of the intact recipient chromosome by some mode of crossing-over. Two modes of transduction are now recognized: in the pneumococcus, the exogenotes consist of a suspension of chemically extracted DNA; in enteric bacteria, the exogenote is carried by a bacteriophage particle. The fragments are so small that only exceptionally are two markers so closely linked as to be transduced together.

In principle, either intact chromosomes or intact nuclei must also be considered as alternative units of exchange in transduction experiments, especially, since nuclei have been successfully transplanted by artificial means.[12]

The most explicit claim of transduction outside the bacteria has been that the tumorigenic quality itself may be transduced by DNA preparations.[13] Subsequently, however, Klein[14] placed suitable immunogenetic markers on the donor and recipient cells, and showed complete linkage of these with the neoplasia. Whatever this case may prove to be, it is therefore not a simple transduction. That is, the unit of exchange is more than a fragment. It remains to be verified whether it is less than a cell. A combination of physical, enzymatic (DNAase) and genetic studies should help to clarify this issue. Klein's example should encourage the intelligent use of genetic markers in other situations where one must exclude the persistence of intact cells.

These examples should stimulate many efforts at achieving transduction in tumor systems, a result that would add a powerful technique for genetic analysis. As suitable materials become available, both DNA and viruses of limited cytopathogenicity should be tried as possible vectors of exogenotic fragments.

Sexual recombination. While the sexual act of nuclear fusion has become surrounded with elaborate secondary paraphernalia in the course of evolution, the modes of its occurrence in microorganisms[15, 16] justify speculations as to the potentiality for sexual cycles in "somatic" cells. Scattered observations can be adduced for support. The decision will rest on properly designed experiments. Fusion of cells, however, has been indicated in liver cells.[17] The same authors have also described the confluence of nuclei or of metaphase spindles. Segregation is more problematical. Somatic segregation and crossing-over has been extensively studied in fruit flies[18] and in plant and microbial materials. Mosaic patches have been described from time to time in heterozygous birds and mammals,[19] but it has not yet been possible to ascribe these to chromosome or gene loss, or to a more regular process of segregation. It is hopeful, however, that somatic variegation (segregation?) is accelerated by radiations in birds[20] as well as in fruit flies[21] and microorganisms.[22, 23] Whatever the mechanism of these variegations, they still allow definite hope of diagnosing recessive genes in heterozygous organisms, if the variegation can be experimentally controlled. Since the unit processes are rare, we may have to rely on selective genetic methods; *a priori*, the hope of success is hardly less than it was for bacteria.[11, 15]

Both transduction and sex should be visualized as aspects of genetic recombination. The most suitable markers may be those that can be independently studied by breeding tests in the intact mouse, outstandingly the histocompatibility factors. Other markers currently available are only drug resistance and the ascites adaptation. We may already have some hint of recombination in studies of the effect of F-1 hybrid hosts on the histocompatibility patterns of transplanted tumors,[8, 24] if this does prove to be more than selection of spontaneous variants. The hybrid host is unique in being compatible with, but genetically distinct from, the tumors. If this effect is recombinational, it may help to use lines that are genetically better defined and, perhaps, to use additional markers such as drug resistance.

All recombination studies with tumor cells suffer from the burden of working with diploid, even polyploid material, which of course complicates the detection of recessive markers. A worthwhile preliminary to these studies would be the surveillance of *heterozygous* tumors, preferably derived from and maintained in F-1 hybrids of Snell's well-defined isogenic-resistant lines, to verify the occurrence and control of possible segregation, as might be revealed by occasional takes in the parental lines.

It would be difficult to estimate the importance of the accomplishment, the possibility of exact genetic analysis of tumors and tissues. Nothing less is likely to resolve the genetic bases of differentiation and of neoplasia.

Virus infection.[11, 25] Studies with lysogenic bacteria and bacteriophages have lately verified an old speculation, familiar to students of cancer viruses, that virus infection is also a species of genetic recombination. First of all, at least one virus, lambda, in *Escherichia coli* K-12 is associated with a specific nuclear site: crosses of lysogenic with sensitive bacteria show that this property segregates in the same fashion as any other mutation, being linked to a locus for galactose fermentation. But, without further evidence, it should not be assumed that other symbiotic viruses do or do not have a locus in the nucleus. Second, the mere presence of a symbiotic virus sometimes confers a unique property on the bacterium that carries it. The new property may relate directly to virus infection or it may concern the formation of exotoxin in diphtheria, or the somatic antigen in group E *Salmonella.*

Cytoplasmic factors.[26, 27, 28] Extranuclear genetic factors (plasmids) have not yet been definitely ascertained in bacteria, but are important features of the genetics of other microorganisms. So far, it has not been possible, for example, to reintroduce chloroplasts into albino green plant cells, or respiratory granules into cytochrome-oxidase-deficient yeast, by exogenous "infection." Whether mitochondria have genetic functions has been debated. For this reason, Lettré's account[29] of the reconstitution of ascites tumor cells that have been deprived of their mitochondria is of extraordinary genetic interest, as it would open the possibility of studying heterologous combinations of mitochondria and cells, a new type of genetics. Regrettably, this work has not yet been subjected to independent confirmation. When it is, genetic markers should be used to exclude the persistence of intact viable cells that otherwise escape notice, as well as to explore genetic diversification of the cytoplasmic granules.

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